

CALORIE CONTROL COUNCIL

Comments on Draft NTP Technical Report GMM 1:

“NTP Technical Report on the Toxicity Studies of Aspartame (CAS No. 22839-47-0) In FVB/N-TgN(v-*Ha-ras*)Led (Tg.AC) Hemizygous Mice and Carcinogenicity Studies of Aspartame In B6.129-*Trp53*^{tm1Brd} (N5) Haploinsufficient Mice (Feed Studies)”

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These reviewers are pleased to note the history of the NTP in publishing study results in the peer-reviewed scientific literature. These results reporting the evaluations of a widely used sweetener in novel transgenic models is of great interest to the scientific community as well as the general public. We believe it is very much in the scientific and public interest for the NTP to incorporate these data fully into the complete body of work establishing the safety of aspartame. Additionally, we encourage the peer-reviewed publication of these results when this NTP Report is finalized. It is with these objectives in mind that we are compelled to offer the following comments on this NTP Draft Report.

These comments are provided to address issues noted in this NTP Draft Report. Comments fall into four categories:

- Incomplete reporting of the study data as described in Section 1.
- Errors of fact contained within the NTP Draft Report that should be corrected or otherwise verified for accuracy. These are described in Section 2.
- Selective citations of the scientific literature are noted in Section 3.
- Misleading statements that can result in erroneous conclusions on the part of the reader are noted in Section 4.

Section 1 -- Incomplete Reporting of the Study Data

Incomplete: Draft Report Title, Cover Page and Page 1

The title of the report does not appropriately reflect the entire scope of the research performed. In particular, the report title does not reflect that this report provides the first data evaluating a negative control in the B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient strain of transgenic mice (commonly referred to as the “p16” strain). These initial negative control data from this transgenic strain are critically important in that the NTP has underway “...studies of two known rodent carcinogens, phenolphthalein and glycidol, and of the known human carcinogen benzene...” (NTP Draft Report GMM 1, page 67, lines 5-7). As data from these positive control compounds become available for the B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient strain, the availability of negative control data for the B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient strain should be apparent from the title of this NTP Final Report.

Suggested title inclusive of the research accomplished: “NTP Technical Report on the Toxicity Studies of Aspartame (CAS No. 22839-47-0) In FVB/N-TgN(v-*Ha-ras*)Led (Tg.AC) Hemizygous Mice and B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient Mice and Carcinogenicity Studies of Aspartame In B6.129-*Trp53*^{tm1Brd} (N5) Haploinsufficient Mice (Feed Studies)” [suggested change underscored]

Incomplete: Abstract, Page 5, Lines 8-9

The “p16” B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient study was completed and is included in the Discussion section as well as in Appendix I. Although the relevance of this model is properly questioned in the report, this study was completed, and the results should be mentioned in the abstract, along with those from the other transgenic strains.

Suggested revision inclusive of the results reported: “This report focuses on the findings in the Tg.AC hemizygous and p53 haploinsufficient mouse models and also includes results from the B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient mouse model.” [suggested change underscored]

Incomplete: Abstract, Page 6

Only the Tg.AC and p53 models are mentioned. The B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient mouse model (p16) was completed, and thus, the results should be included as for the other two models, one of which (Tg.AC) was also in retrospect questioned for its usefulness for assessment of carcinogenicity.

Incomplete: Conclusions, Page 7

Conclusions should represent the results of all three studies.

Suggested revision: “Under the conditions of the 9-month feeding studies, there was *no evidence of carcinogenicity of aspartame** in male or female p53 haplotypeinsufficient mice, Tg.AC hemizygous mice, or B6.129-Cdkn2a^{tm1Rdp}(N2) Deficient mice. [suggested change underscored]

Incomplete: Appendix I, Page I-4

Aside from any issue of relevance of the “p16” B6.129-Cdkn2a^{tm1Rdp} (N2) Deficient mouse model, important negative control data were collected in the p16 strain of transgenic mice, and the results of the evaluations of the carcinogenicity and tumor incidence data should be provided in this section of the NTP Final Report. Because the NTP has yet to report the results of a number of positive control compounds in this p16 model, it is important to report the tumor incidences in this potentially important strain of transgenic mice after nine months of dietary dosing with aspartame.

Section 2 -- Errors of Fact**Error: Abstract, Page 5, Lines 2-3**

The statement, “a small number of neoplasms of the brain were observed in a rat study” is an error of fact concerning aspartame, as it does not accurately represent the data or the conclusions of the detailed evaluations of the carcinogenicity data. Brain tumors occurred in control groups as well as treated groups. When the data from these studies were appropriately analyzed by sex and dose group using internationally accepted standards of analysis, there was clearly no effect of aspartame on brain tumors in rats in any study.

Based on detailed evaluations of the carcinogenicity data, cancer experts in major regulatory agencies and expert committees around the world including the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 1980, 1981), the US FDA (1981, 1996), the Health Protection Branch of Canada (HWC, 1981), the Committee on Toxicity of Chemicals in Food, Consumer Products, and the Environment (COT) of the UK Ministry of Agriculture, Fisheries, and Food (MAFF, 1982), the EU Scientific Committee on Food (SCF, 1985, 1997, 2002), the Australia New Zealand Food Authority (ANZFA, 1997), the UK Committee on Carcinogenicity (COC) Department of Health (1998), the French Food Safety Agency (2002), and Health Canada (2003), all concluded that aspartame does not cause cancer, including brain cancer.

Error: Abstract, Page 5 & Introduction, Page 13 (same error both places)

Regarding Trade Names: NutraSweet is the only trade name for aspartame; aspartame is most widely available under the common name.

The statement is made that Canderel, Equal, Sanecta, and Tri-Sweet are trade names for aspartame; this is not correct. These are trade names for tabletop sweeteners that contain small amounts of aspartame as the sweetening ingredient. For example, tabletop sweeteners in sachet form are only about 4% aspartame by weight.

Error: Introduction, Page 13, Line 4

Aspartame is about 180 to 200 times sweeter than sucrose depending on the formulation and food matrix (Stegink, et al., 1977) – not 160 as stated in text. Reference for the '160' figure is not clear.

Error: Introduction, "Production, Use, and Human Exposure," Page 14, Line 16

The statement, "average daily intake of aspartame may be 3 to 5 mg/kg body weight" is either incomplete or incorrect. These numbers do not represent average or mean daily consumption of aspartame in the US. As stated in the references cited in this NTP Draft Report, these numbers represent the daily consumption of heavy users (*90th percentile*) of aspartame for either the general population (3 mg/kg bw/day) or children (~5 mg/kg bw/day) (Butchko and Stargel, 2001).

Error: Introduction, "Absorption, Distribution, and Excretion," Page 16, Line 13

The statement, "Uncontrolled phenylketonurics have phenylalanine plasma levels *of up to* [emphasis added] 1200 μ M" is not correct. Uncontrolled PKU individuals have plasma phenylalanine concentrations *greater than* 1200 μ M (Caballero et al., 1986; Wolf-Novak et al., 1990; Mackey and Berlin, 1992).

Error: Introduction, "Absorption, Distribution, and Excretion," Page 16, Line 18

The word "aspartame" in the phrase "...aspartame plasma level of phenylalanine..." is incorrect and should be deleted. Aspartame is not absorbed intact (Ranney et al., 1976; Oppermann, 1984; Stegink, 1984; Butchko et al., 2002, numerous others), thus, an "aspartame plasma level" cannot be measured. However, the plasma concentrations of the products of aspartame metabolism (simple digestion) can be measured, and those are all normal dietary constituents.

Error: Introduction, "Toxicity, *Humans*," Page 17, Last line on page

The use of the term "side effects" in the phrase "system for reporting side effects" is incorrect in this context, as it implies a cause and effect relationship. Such relationships cannot be established based on anecdotal data, and thus no "side effects" of aspartame were established. A phrase here that would be correct and would not mislead the reader might be "system for collecting and evaluating anecdotal reports."

Error: Introduction, “Toxicity, Humans,” Page 18, Line 2 and Line 19

The term “side effects” is incorrect in the context used as described in the previous comment. It would be more appropriate to substitute the word “reports” for “side effects reported” on Line 2 and “anecdotal reports after” for “side effect from” on Line 19.

Error: Introduction, “Toxicity Humans,” Page 18, Line 13

The statement that the CDC “...published an evaluation of *reactions* [emphasis added] to aspartame...” is incorrect. The CDC (1984) published an evaluation of “complaints” that were not verified as “reactions” to aspartame.

For accuracy, any discussion of the postmarketing surveillance of anecdotal reports with aspartame should include the overall conclusions of the FDA and CDC – that the reports were generally mild, were symptoms common in the general population, no “constellation of symptoms” could be related to aspartame use, and “focused” clinical studies were the only way to evaluate the issues thoroughly (CDC, 1984; Bradstock et al., 1986; FDA, 1995; Tollefson, 1988; Tollefson et al., 1988; Tollefson and Bernard, 1992; Tollefson, 1993).

Error: Introduction, Toxicity. Humans,” Page 19, Line 12

The word “injection” should be “ingestion.”

Error: Introduction, “Toxicity Humans,” Page 19, Line 17

The attribution that FDA said “people with phenylketonuria...” “...should not use aspartame (FDA, 1994)” is not found in the citation provided. The cited reference does say that aspartame is a source of phenylalanine and should be labeled as such for information purposes for individuals with phenylketonuria (PKU).

Error: Introduction, “Reproductive and Developmental Toxicity, Experimental Animals, Page 20, Lines 5-8

The implication that study data and dose levels were not available from two-generation reproductive, perinatal, and postnatal toxicity studies done by G.D. Searle & Company is not correct. Summaries of independent evaluations, including methods, results, discussions, and conclusions, have been published for these studies by JECFA (WHO, 1980). All safety data on aspartame, including entire studies and all data, are publicly available upon request from the FDA.

Error: Introduction, “Carcinogenicity, Experimental Animals,” Page 21, Lines 5-8

The statement that “...the experimental details of the [carcinogenicity] studies were not published...” is not correct. Experimental details of these studies are available in JECFA (WHO, 1980, 1981), Ishii (1981), Ishii et al. (1981), Cornell et al. (1984), Koestner (1984), and Butchko et al. (2002).

All safety data on aspartame, including entire studies and all data, are publicly available upon request from the FDA.

Error: Introduction, “Carcinogenicity, *Experimental Animals*,” Page 21-22

The implication from this section is not correct that US FDA may be the only major regulatory body to ever evaluate carcinogenicity studies with aspartame. As stated in the previous comment, the implication from this section that experimental details of these studies are not available is not correct; they are available in JECFA (WHO, 1980, 1981), Ishii (1981), Ishii et al. (1981), Cornell et al. (1984), Koestner (1984), and Butchko et al. (2002). All safety data on aspartame, including entire studies and all data, are publicly available upon request from the FDA.

Error: Introduction, “Carcinogenicity, *Experimental Animals*,” Page 21-22

The statement in the sentence starting on the last line of page 21 and concluding on the first line of page 22 is egregious and scientifically bizarre. The statement regarding a 104-week rat study with aspartame that, “A total of seven brain tumors occurred in exposed males, and a total of five occurred in females; one occurred in a control male,” is NOT a statistically or scientifically valid approach for reporting tumor incidence spread across different dose groups. Accurate tumor incidences by sex and dose group have been published elsewhere for this and other carcinogenicity studies done with aspartame (FDA, 1981; WHO, 1980, 1981; Butchko et al., 2002).

In total, three two-year rat studies and one two-year mouse study evaluated the carcinogenic potential of aspartame, and two two-year rat studies and one two-year mouse study evaluated the carcinogenic potential of the diketopiperazine (DKP) of aspartame (WHO, 1980, 1981; FDA, 1981, 1983; Ishii, 1981; Ishii et al., 1981; Kotsonis and Hjelle, 1996; Butchko et al., 2002). Each of these studies identified no evidence of carcinogenicity of aspartame or its diketopiperazine.

The source for the corruption of carcinogenicity data repeated in this NTP Draft Report was an insupportable manipulation of the data from one of these seven rodent studies (E 33/34). Specifically, in the 1970s a long-time critic of aspartame combined brain tumor data from both sexes and *independent* dose groups to create an apparent dose-response relationship for study E 33/34. The term “long-time critic” is applicable in that this individual had attempted to link aspartame consumption to potential neurotoxicity without success or scientific acceptance for a number of years prior to this corruption of carcinogenicity data. Thus, this flawed post hoc analysis arrived in the 1970s with the potential of bias.

The actual brain tumor data in this study (E33/34) are as follows:

**Brain tumors in the 2-year carcinogenicity study with aspartame in
Sprague-Dawley rats (E-33/34)^{a,b} (From Table 2, page S69, Butchko et al., 2002)**

Dose (mg/kg/day)	Number of males	Number and type of brain tumors in males	Number of females	Number and type of brain tumors in females
0	59	1 (astrocytoma)	59	0
1000	36	1 (astrocytoma) 1 (astrocytoma)	40	1 (astrocytoma) 1 (astrocytoma)
2000	40	1 (astrocytoma)	40	0
4000	40	1 (oligodendroglioma) 1 (astrocytoma) 1 (astrocytoma) 1 (astrocytoma)	40	1 (astrocytoma)
6000-8000	39	0	38	1 (medulloblastoma) ^c 1 (astrocytoma)

^a From FDA, 1981, E-33/34, E-87 (Innes), and E-102 (UAREP).

^b E-33/34 was originally evaluated with two sections per brain but was re-assessed with eight sections per brain by Innes (E-87) and then by UAREP (E-102).

^c Animal died during week 13 of the study (about 16 weeks of age); this medulloblastoma was considered to be embryonal in origin and not related to treatment.

Specifically, the data were corrupted by combining tumor incidence from males and females in the two independent low dose groups and then contrasted the results with combined tumor incidence data from the two independent higher dose groups to achieve the appearance of a dose response. Thus, the random distribution of brain tumors for combined sexes in this study of 1, 4, 1, 5, 2 (Control, 1000, 2000, 4000, and 6000-8000 mg/kg/day dose groups, respectively) (FDA, 1981) was manipulated into an incidence for combined sexes equivalent to “1, 5, 7” for the control, two lower, and two higher dose groups, respectively. The appropriate and accepted evaluation of carcinogenicity studies includes analysis of tumor incidence data by sex and independent dose groups compared against concurrent controls. When the data were analyzed according to standard and accepted practices -- by sex and dose groups -- there is no evidence of carcinogenicity with aspartame.

However, in this Draft Report, NTP now bizarrely proposes to *further* corrupt the data from E-33/34 by separating genders while commingling ALL the tumor incidence data from ALL the independently dosed groups: The NTP Draft Report states (last line of page 21 continuing on to the first line of page 22), “A total of seven brain tumors occurred in exposed males, and a total of five occurred in females; one occurred in a control male.” While this statement may be technically correct, the analysis is not only misleading but is scientifically and statistically *indefensible*. Further, such manipulations of data from independent dose groups are scientifically and statistically *meaningless* according to internationally accepted standards. Consequently, such data manipulations should not be repeated or practiced by NTP.

The results of a second long-term bioassay in rats with *in utero* exposure (E-70) are as follows:

Brain tumors in the 2-year carcinogenicity study including *in utero* exposure with aspartame in Sprague-Dawley rats (E-70)^{a,b} (Table 3, page S69, Butchko et al., 2002)

Dose (mg/kg/day)	Number of males	Number and type of brain tumors in males	Number of females	Number and type of brain tumors in females
0	58	1 (astrocytoma) 1 (astrocytoma) 1 (astrocytoma)	57	1 (astrocytoma)
2000	36	1 (ependymoma) 1 (astrocytoma)	39	1 (astrocytoma)
4000	40	1 (astrocytoma)	40	1 (meningioma)

^a From FDA, 1981, E-70, E-87 (Innes), and E-102 (UAREP).

^b Eight sections per brain were evaluated.

This study was faulted by the critic because the incidence of brain tumors in concurrent controls exceeded the same long-time critic's expectations (FDA, 1981). However, the overall incidence of brain tumors was approximately 3% in both E-33/34 and E-70 (FDA, 1981; Koestner, 1984, 1997; Butchko et al., 2002). This incidence is well within the expected ranges for brain tumors in Sprague-Dawley rats (Swenberg, 1986), especially considering the additional microtumors detected in aging rats after the large number of brain sections examined in these aspartame studies (8 versus the current standard of 3).

The brain tumor incidence from a third two-year study done in Wistar rats is as follows:

Brain tumors in the 2-year carcinogenicity study with aspartame and diketopiperazine (DKP) in Wistar rats^{a,b} (Table 4, page S70, Butchko et al., 2002)

Dose (mg/kg/day)	Number of males	Number and type of brain tumors in males	Number of females	Number and type of brain tumors in females
Control	59	0	60	1 (atypical astrocytoma)
1000	59	1 (oligodendroglioma)	60	0
2000	60	0	60	1 (astrocytoma) 1 (ependymoma)
4000	60	1 (astrocytoma)	60	0
3000 + 1000 DKP	60	0	60	1 (oligodendroglioma)

^a From: Ishii, 1981.

^b Six sections per brain were evaluated.

There was no evidence of an effect on brain tumors after dosing with either aspartame or DKP in any of the carcinogenicity studies in rats or mice.

After this large amount of rodent carcinogenicity data was evaluated according to standard and internationally accepted criteria, scientists within regulatory bodies around the world, including the US FDA (1981), the Canadian Health Protection Branch (HWC, 1981), the UK Committee on Toxicity of Chemicals in Food, Consumer Products, and the Environment (MAFF, 1982), and the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1980, 1981), concluded that aspartame is not a carcinogen. Characterizations to the contrary clearly are not supported either by the facts themselves or the conclusions of scientists who have evaluated the actual data independently and in its entirety.

The discussion of the issues regarding the long-term and lifetime carcinogenicity studies with aspartame in this NTP Draft Report, including allusion to “brain tumor diagnosis inconsistencies” (Page 22, Line 2) is incomplete and misleading. The different evaluations of this vast amount of carcinogenicity data by various expert groups clearly confirmed “consistencies” in the diagnoses rather than “inconsistencies.” Consequently, it is not clear why incidental “inconsistencies” are highlighted in this NTP Draft Report.

Most critical, and not reflected in this section or elsewhere in this NTP Draft Report, is that this issue was carefully evaluated and resolved by experts within academia, at major regulatory bodies, and on expert committees both prior to and after the numerous approvals of aspartame for human use, e.g., the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1980, 1981), FDA (1981), Health Protection Branch of Canada (HWC, 1981), UK Committee on Toxicity of Chemicals in Food, Consumer Products, and the Environment (MAFF, 1982), Koestner (1984, 1997), Cornell et al. (1984), the EU Scientific Committee on Food (SCF, 1985, 2002), the Australia New Zealand Food Authority (ANZFA, 1997), Flamm (1997), the French Food Safety Agency (2002), Butchko et al. (2002) and many others. Failure to acknowledge these efforts and conclusions is hurtful to the hundreds of independent experts who over the last thirty-plus years have reviewed and evaluated the carcinogenicity database for aspartame in its entirety.

This section should be corrected in order for this NTP Draft Report to maintain integrity and scientific objectivity.

Error: Introduction, “Carcinogenicity, *Humans*,” beginning Page 23

The statement is not correct that “one study reported... an increase in brain tumors in the US from 1970 – 1980...” [emphasis added]. Specifically, the same long-time critic responsible for misrepresenting animal brain tumor data in the 1970s alleged an increase in brain tumor incidence in humans in the US in the *mid-1980s*, a few years after aspartame was marketed. The association between aspartame and brain tumors (Olney et al., 1996) was based on a selective presentation of epidemiological data from the US Surveillance, Epidemiology and End

Results (SEER) tumor database maintained by the National Cancer Institute (NCI). Inexplicably omitted from the critics' analysis of SEER data were entries for the years 1973 and 1974 which, had they been included, would demonstrate that a trend towards increased brain tumors *predated* aspartame approval (Levy and Hedeker, 1996).

Further, granting scientific credibility to the new allegations from this critic requires two biologically implausible assumptions: first, that a certain factor (aspartame) could cause an increase in the incidence of brain cancer in less than 4 years (aspartame was not available in the US until late 1981 when it was approved for dry uses and not widely used until after approval in beverages by FDA in mid-1983) and second, that even more widespread exposure to this factor would cause no further increase in tumor incidence in subsequent years (Butchko et al., 2002). In actuality, overall brain tumor rates appear to be *decelerating* (Levy and Hedeker, 1996). The SEER report providing data for 1973 to 1996 (NCI, 1999) shows that the overall percent change in incidence of brain tumors increased 1.6% between 1975 and 1979 before aspartame was marketed, but *decreased* 6.6% between 1992 and 1996 after aspartame was marketed. For these same intervals, the SEER database reports an annual percent change of 0.6% for 1975-1979 vs. -2.2% for 1992-1996, a statistically significant decrease for the most recent interval.

Malignancy of brain tumors was further purported to have increased after marketing of aspartame (Olney et al., 1996). If this were true, the overall mortality from brain tumors would be expected to rise. To the contrary, the percent change in mortality for all ages *decreased* 2.1% from 1992 to 1996 (it had *increased* 2.5% from 1975 to 1979). Further, relative 5-year survival rates after brain tumor diagnosis were significantly higher from 1989 to 1995 compared to 1974 to 1976 (NCI, 1999). Again, declining mortality is not consistent with a true increase in brain tumors or an increase in malignancy of brain tumors (Butchko et al., 2002).

Since the alleged increase in human brain tumors cited in this NTP Draft Report appeared, peer-reviewed publications, including by researchers within the NCI, have pointed out that changes in SEER data during the mid-1980s can be explained largely by changes in the classification system for brain tumors, the ability to diagnose tumors earlier with advanced neuroimaging techniques, and changes in neurosurgical practices, such as the availability of stereotactic biopsies, all of which took place during the mid-1980s (for details and references, see Butchko et al., 2002). Thus, the NTP Draft Report provides unwarranted credibility to "these findings" when it states "other factors could better account for *these findings* [emphasis added]" (Page 23, Line 13).

For completeness on this issue, the NTP report should also cite scientists at regulatory and government bodies in several countries that have evaluated the allegations of Olney et al. (1996). Among their conclusions:

"A recent analysis of the NCI statistics on cancer incidence in the United States does not support an association between the use of aspartame and an increased incidence of brain tumors," (NCI, 1997).

The analysis "does not support an association between the use of aspartame and increased incidence of brain tumors," (FDA, 1996).

The Committee on Carcinogenicity "...expressed serious concerns about the quality of the paper and concluded that it did not raise any concerns with regard to the use of aspartame..." in the UK (MAFF, 2000; Caseley and Dixon, 2001).

"...the data do not support the proposed biphasic increase in the incidence of brain tumours in the USA during the 1980s," (The EU Scientific Committee on Food [SCF], 1997).

"From the extensive scientific data available at this stage, the evidence does not support that aspartame is carcinogenic in either animals or humans. There appears to be no foundation to recent USA reports of increased brain tumors in humans," (the Australia New Zealand Food Authority [ANZFA], 1997).

"None of the carcinogenicity tests that have been conducted on rodents indicated a relationship between treatment with aspartame and the appearance of brain tumours. The epidemiological study by Olney et al. which suggested a link between the placing on the market of aspartame and a possible increase in the frequency of brain cancers in humans did not provide any scientific evidence to justify or demonstrate a basis for this suggestion; to date it has not been confirmed," (French Food Safety Agency [AFSSA], 2002).

Error: Study Rationale, Page 28, Line 17-18

The statements, "It has been argued that those *studies* [emphasis added on plural] showed a slight increase in brain tumors. Because of these *uncertainties* [emphasis added], aspartame was selected by the NIEHS for evaluation of its toxicologic and carcinogenic potential," are incorrect and are an invalid rationale to do additional cancer studies with aspartame. The only individuals to have argued "a slight increase in brain tumors" were Olney et al. (1996) after corruption of non-dose-related data from independent dose groups in *one rodent study*, critics who published their opinions on the Internet, and a few individuals with little or no familiarity with the actual database on rodent carcinogenicity testing with aspartame or expertise in animal carcinogenicity testing.

Further, to characterize the results of earlier studies as having left "uncertainties" is to distort the facts and the conclusions of experts who have evaluated the actual studies and who are most familiar with the data, such as at FDA (1981), HWC (1981), JECFA (WHO, 1980, 1981), the Committee on Toxicity of Chemicals in Food, Consumer Products, and the Environment (COT) of the UK Ministry of Agriculture, Fisheries, and Food (MAFF, 1982), SCF (1985, 2002), UK Committee on Carcinogenicity

(Department of Health, 1998), National Cancer Institute (1997) and others. The only obvious scientifically defensible rationale for additional studies with aspartame in non-validated animal models of cancer would be as a negative control, as was the stated rationale for testing acesulfame-potassium in two of these mouse models (Draft Technical Report NTP GMM 2, page 18, last line). Clearly scientists within NTP, being aware of these publicly available scientific data and evaluations, could only have intended to use aspartame as a negative control in evaluating these novel strains of transgenic mice.

Error: Discussion and Conclusions, Page 63, Line 3

The phrase, “no *consistent* [emphasis added] carcinogenic response” would be more correctly written as, “no evidence of carcinogenic response in any study.”

Error: Discussion and Conclusions, Page 63, Line 4

The phrase, “lingering concern” in regards to brain tumors with aspartame is irresponsible, egregious, and without scientific support. All matters related to brain tumors and aspartame have been resolved. Other than one long-time critic of aspartame, any questions and concerns have come from the Internet and a few people not familiar with the data or the conduct and evaluation of carcinogenicity studies in animals and their followers. Worldwide regulatory authorities would not allow a material to be marketed with “lingering concern” regarding carcinogenicity. Minimally, the term “lingering concern” should be clarified as to whom any concern did linger. As discussed earlier, there is *no* lingering concern in the minds of international cancer experts, worldwide regulatory agencies, or anyone objectively familiar with the publicly available evidence.

Section 3 -- Incomplete or Selective Citation of the Literature

Two grievous oversights in the cited literature for aspartame safety are the extensive reviews and evaluations published by WHO (1980, 1981), and a recent extensive review of safety of aspartame authored by 24 scientists from 19 institutions in five countries. Other examples of more appropriate or more complete literature citations are summarized below:

**Selective: Introduction, “Absorption, Distribution, and Excretion,”
Page 14-16**

Scientific citation within this section is extremely selective and does not reflect the extensive number of studies that have been done to evaluate aspartame metabolism (simple digestion) and the disposition of its components in humans. The NTP Draft Report does not reference or discuss these numerous studies. These numerous published studies have been reviewed in a number of sources including WHO (1980), Stegink (1984), others and most recently Butchko et al. (2002).

Selective: Introduction, Page 16, Line 3

Methanol, a normal component of the human diet, has long been known to be oxidized to formaldehyde and formic acid after ingestion. There is nothing novel or original regarding this observation in the reference cited, Trocho et al. (1998). The metabolism of methanol to formate is well covered in the paper cited in the line above (page 16, line 2), Ranney et al., 1976. Further, the paper cited (Trocho et al., 1998) has been criticized for the authors' apparent lack of familiarity with usual normal disposition of methanol in the body (Tephly, 1999).

Selective: Introduction, "Toxicity, *Experimental animals*," Page 17

This section is very selective by citing only six references. The section does not reflect or make any attempt to summarize the vast amount of publicly available animal toxicity data on aspartame. Independent evaluations of toxicity data in experimental animals has been provided by WHO (1980), FDA (1981), and other regulatory bodies, and reviews of these studies are available from Molinary (1984), Kotsonis & Hjelle (1996), and Butchko et al. (2002).

Selective: Introduction, "Toxicity, *Humans*," Page 18-19

The discussion of the scientific studies with aspartame in humans is inadequate and is apparently based on an incomplete and selective citation of the scientific literature. The extensive database of human studies with aspartame has been reviewed by JECFA (WHO 1980), Tschanz et al. (1996), Butchko et al. (2002), SCF (2002) and many others not cited in the NTP Draft Report.

For example, regarding headaches, the NTP Draft Report discusses an outpatient study by Van Den Eeden et al. (1994) from which the authors claimed "some people were susceptible to headaches after aspartame consumption." However, the Draft Report does not cite published statistical criticisms of this study and the fact that the study outcome can be largely accounted for by the results from one outlier subject out of 27 subjects (Levy et al., 1995). The Draft Report does not cite another study done under the controlled conditions of a clinical research unit that concluded that aspartame was no more likely to cause a headache than placebo (Schiffman et al., 1987).

Selective: Introduction, "Toxicity, *Humans*," Page 19

The NTP Draft Report cites a study by Nguyen et al. (1998) alleging that "injection" (actually ingestion) of a *single* dose of 250 mg of aspartame by healthy subjects increased urinary calcium excretion. However, Leon (1999) responded to this study with data from his study (Leon et al., 1989), which showed *no* effect of aspartame on urinary calcium excretion in 108 healthy people given placebo or aspartame at 75 mg/kg body weight (about 5250 mg/day for a 70 kg adult) daily for 24 weeks.

Selective: Introduction, “Reproductive and Developmental Toxicity, *Experimental Animals*,” Page 19-20

As discussed above for the human studies, there appears to be incomplete and selective citation of the scientific literature on aspartame. The extensive animal reproductive and developmental toxicity studies with aspartame have been reviewed by JECFA (WHO, 1980), Molinary (1984), Kotsonis and Hjelle (1996), and Butchko et al. (2002). Any summary of data in this section should particularly include reference to the extensive summaries and evaluations of the JECFA (WHO, 1980).

Selective: Introduction, “Carcinogenicity, *Experimental Animals*,” Page 22-23

All citations of Molinary (1984) on these pages should also cite WHO (1980, 1981), Kotsonis and Hjelle (1996), and Butchko et al. (2002), where more complete study details and results may be found than in the reference cited in the NTP Draft Report.

Selective: Introduction, “Carcinogenicity, *Humans*,” Page 23, Lines 7-8

Although the NTP Draft Report includes Gurney et al. (1997) in a list of “...others...” “...suggesting other factors...” than aspartame use in the incidence of human brain cancer (Page 23, Line 13), the NTP Draft Report does not recognize that Gurney et al. was an epidemiological study of aspartame in children when in the previous paragraph states, “There have been no large-scale epidemiology studies...” on aspartame, and cites no epidemiological work. Gurney et al. (1997) should be mentioned as an epidemiology study regardless of “scale.” Gurney et al. was a case-control study evaluating aspartame consumption and the risk of primary brain tumors of childhood diagnosed between 1984 and 1991. This epidemiology study published in the *Journal of the National Cancer Institute* found that children with brain tumors were no more likely to have consumed aspartame than were children in the control group. There was also no increased risk from maternal consumption of aspartame during pregnancy, and no evidence of an association of specific types of brain tumors with aspartame consumption.

Selective: Introduction, “Genetic Toxicology,” Page 23, Line 16 through Page 24, Line 4

More than half of the summary on “Genetic Toxicology” (11 of 17 total lines) is devoted to summarizing one study by Shephard et al. (1993). Shepard et al. evaluated nitrosation of a number of peptides/amino acids under non-physiological conditions and identified no mutagenicity unique to aspartame-reaction products versus reaction products of other peptides. Critics have postulated nitrosation of aspartame or DKP as a potential source of carcinogenic compounds and have cited Shephard et al. as a basis for speculation (e.g., Olney et al., 1996). This section of the NTP Draft Report does not cite careful deliberations of regulatory bodies on this question or studies done to evaluate nitrosation of aspartame and the DKP breakdown product of aspartame (WHO, 1980; FDA, 1983;

Butchko et al., 2002). In summary, there is no evidence of stable nitroso-formation with aspartame or its DKP breakdown product under physiological or even severe conditions of nitrosation. This finding is not unexpected since stable nitrosamines form primarily from secondary amines and neither aspartame nor DKP is a secondary amine. Further, for nitrosation to occur there must be a source of nitrite. Nitrite levels are extremely low in foods typically sweetened with aspartame (Flamm, 1997).

In addition to mentioning regulatory deliberations regarding nitrosation of aspartame, the Draft Report should mention that in the article cited (Shephard et al., 1993) the extremely high concentrations of nitrite used reacted with a variety of amino acids and peptides, including aspartame, and generated compounds with apparent mutagenic properties when incubated with bacteria without metabolic activation. The chemical conditions that produced reactive products in Shephard et al. (1993) were neither unique to aspartame nor relevant to aspartame consumption. For example, the nitrite concentrations used were at least 10,000 times greater than human gastric levels. Any potential reaction products of aspartame would be the same as those formed from common dietary proteins, peptides and/or amino acids, or DKPs of peptides, which are present in the diet at far higher concentrations than aspartame. Consequently, nitrosation of aspartame would have no effect on the total body burden of nitrosamines whether they be preformed or formed in the stomach (Flamm, 1997).

Thus, it is not clear why the majority of the discussion of "Genetic Toxicity" in this section is devoted to a single citation having no relevance to aspartame consumption while more relevant findings and expert deliberations are not mentioned.

Selective: Discussion and Conclusions, Page 64, Line 3

For completeness, the citation for Molinary (1984) should be accompanied minimally by WHO (1980), Kotsonis and Hjelle (1996), and Butchko et al. (2002).

Section 4 -- Misleading Statements That Can Result in Erroneous Conclusions on the Part of the Reader

There are a number of statements in the Introduction section of the NTP Draft Report that are misleading and perpetuate false information. A number of these cases are addressed below:

Misleading: Introduction, “Production, Use, and Human Exposure,”

Page 14, Lines 10 –14

The NutraSweet Company is not the only producer of aspartame sold in the US. Aspartame is also sold in the US by other companies, including Ajinomoto, Holland Sweetener Company, and Daesang. The relevance of production methods in this section of the NTP Draft Report is unclear. A number of different methods are used to manufacture aspartame, and these methods are largely proprietary. Thus, this information should be deleted.

Misleading: Introduction, “Absorption, Distribution, and Excretion,”

Page 14, Line 21

Aspartame does not enter the bloodstream (Ranney et al., 1976; Oppermann, 1984; Stegink, 1984; Butchko et al., 2002, numerous others). Thus, to state “hydrolysis occurs during the absorption process” is misleading. As noted in the diagrams on page 15 of the NTP Draft Report, hydrolysis of aspartame into its three components occurs either in the gut lumen or in the mucosal cells lining the GI tract. Aspartame is not absorbed into the bloodstream; rather its components, all usual components of the diet, are absorbed into the bloodstream.

Misleading: Introduction, Page 16, Lines 4-6

The statement that aspartame alters the ratio of plasma levels of phenylalanine to other large neutral amino acids (LNAA) when consumed with carbohydrate is misleading when made out of context. For example, the insulin response from sugar and other carbohydrates consumed *alone* results in equivalent changes in the phenylalanine/LNAA ratio (Martin-Du Pan et al., 1982; Stegink et al., 1987; Wolf-Novak et al., 1990; Burns et al., 1991; Butchko et al., 2002). Thus, small changes in phenylalanine/LNAA ratio after aspartame are not unique to aspartame.

Misleading: Introduction, Page 16, Lines 4-8

Regarding brain levels of phenylalanine and the relationship with plasma levels of other large neutral amino acids, the cited reference, Dews (1987) is a brief summary statement on this topic. This citation is not as recent, extensive, or in depth on the topic as are reviews of research on aspartame and neurochemistry by Lajtha et al. (1994), Schomer et al. (1996), and Butchko et al. (2002).

Further, any possible changes in phenylalanine/LNAA after large doses of aspartame or even carbohydrate notwithstanding, such changes are not relevant as they do not lead to consistent changes in brain chemistry in animals (Lajtha et al., 1994; Schomer et al., 1996) or to changes in brain function in humans, e.g., headaches, seizures, behavior, cognition or mood (numerous references reviewed in Tschanz et al., 1996 and Butchko et al., 2002).

This oversimplified paragraph in the NTP Draft Report is misleading in the implication that consumption of aspartame in any amount could result in the extremely high plasma concentrations of phenylalanine that are found in the rare genetic disease, phenylketonuria (PKU). Specifically, the use of the phrase “This condition is *also* [emphasis added] seen in phenylketonuria” in lines 7-8 is incorrect because plasma levels of phenylalanine capable of interfering with development of the nervous system “...in the fetus and in young children...” (Line 6) can ONLY be observed clinically in individuals homozygous for PKU. This paragraph is misleading because in contrast to the implications here, it is not possible for non-PKU individuals to consume enough aspartame to raise plasma phenylalanine concentrations to those associated with PKU (Stegink, 1984).

Most misleading in the omissions from this paragraph is the absent perspective of the amount of phenylalanine children and adults get from diet compared to that from consumption of aspartame. For example, six times more phenylalanine is provided in a glass of non-fat milk than in an equivalent volume of soft drink sweetened with aspartame. Aspartame consumption by a four-year old child at the 90th percentile daily intake of aspartame provides 2.6 mg/kg of phenylalanine per day (derived from Butchko et al., 2002); the *average* daily dietary intake of phenylalanine (not the 90th percentile) by a four-year old child is approximately 206 mg/kg (Stegink, 1984).

**Misleading: Introduction, “Absorption, Distribution, and Excretion,”
Page 16, Lines 9-11**

The statement that “complete absorption and toxicokinetic data are not available to compare disposition of aspartame in humans and animals over the same dose range” is misleading. Doses can be administered to animals in safety studies that cannot be dosed to people. Exaggerated dose levels are used in animal safety studies to demonstrate safety and toxicity. Digestion converts aspartame into its basic components, phenylalanine, aspartate, and methanol, in animals and humans. These components are all normally found in the diet. In contrast to the implications of the statement in the NTP Draft Report, metabolism of aspartame and its components, have been well characterized in animals and humans (Oppermann, 1984; Stegink, 1984; Stegink and Filer, 1996 and many others). The nature of testing non-toxic food additives results in extremely exaggerated doses in animal species, as was the case when NTP dosed these studies at 5% of diet. Further, radiolabelled studies with aspartame (Ranney et al., 1976) in animals and humans demonstrate that aspartame metabolism (simple digestion) and disposition is similar across species.

Misleading: Introduction, “Absorption, Distribution, Excretion,” Page 16, Lines 14 –17

The very large doses of aspartame given in the studies described here should be put into perspective. The reader otherwise could be misled by not having any perspective of doses versus intake. For example, the dose of 34 mg/kg body weight dose is more than 10 times the actual 90th percentile aspartame intake in the general population (Butchko et al., 2002). Also there is no perspective placed on the peak plasma phenylalanine concentrations presented here. For example, even after this very large bolus dose of aspartame, peak plasma phenylalanine concentration remained within the normal postprandial range (Stegink et al., 1977; Stegink and Filer, 1996). Consequently, the phenylalanine concentrations in this paragraph are meaningless without also explaining to the reader their relevance in terms of normal ranges of phenylalanine in humans.

Misleading: Introduction, Page 17, the Section on “Toxicity, *Humans*”

It is misleading to say, “There have been no large-scale, controlled studies to evaluate whether aspartame causes toxic effects in humans.” It is unclear what the authors mean by “large-scale.” From a compliance standpoint, humans simply cannot ingest large numbers of capsules (required in double-blind, placebo controlled studies) of test substances over years. Further, in the vast majority of human studies completed, the appropriate sample sizes were determined by statisticians to have adequate statistical power to enable the investigators to come to the conclusions stated. In addition, by giving exaggerated doses to humans, subjects are exposed to many times what people actually consume.

Before the approval of aspartame, tolerance studies up to 27 weeks in duration were done in healthy adults and children, obese individuals, diabetics, and PKU heterozygous individuals. Hundreds of subjects participated in these studies, which were largely randomized, double-blind, placebo controlled studies. The results clearly demonstrated no adverse health effects of aspartame (these studies are reviewed and referenced in WHO, 1980; Visek, 1984; Tschanz et al., 1996; Butchko et al., 2002, others). In addition, after marketing, a 24-week randomized, double-blind, placebo controlled, parallel designed study was done with over 100 healthy adults at the University of Minnesota using daily doses of aspartame of 75 mg/kg body weight or placebo (Leon et al., 1989). This dose is equal to an adult’s consumption of about 25-30 12-ounce cans of diet beverage with aspartame daily, an amount well outside normal intake patterns. The results of this study confirmed the results of the earlier studies that aspartame is not associated with adverse health effects.

Misleading: Introduction, Toxicity *Humans*,” Page 18, Line 18

Regarding clinical research with aspartame, the statement, “Several studies in the literature have evaluated a *small* [emphasis added] number of people...” is misleading given the many hundreds of subjects evaluated

in clinical studies on aspartame tolerance (e.g., Leon et al., 1989 and others reviewed in WHO, 1980) and the large number of subsequent studies, including the “focused” studies suggested by CDC (reviewed in Butchko et al., 2002; SCF, 2002). A number of clinical studies were undertaken to address issues raised about aspartame. This section of this NTP Draft Report may lead the reader to a false impression to the contrary. A published monograph is entirely devoted to reviewing the many clinical studies with aspartame (Tschanz et al., 1996).

In addition, regarding the “small numbers” of people evaluated, the sample sizes in these studies were carefully selected by statisticians to assure adequate statistical power to answer the questions. In the “focused” clinical studies, subjects were chosen from individuals who were convinced that aspartame caused their symptoms, thus increasing the probability of finding positive responses; there were none. To refer to “*several studies* [emphasis added]” evaluating “*a small number of people* [emphasis added]” ignores the numerous academic experts who conducted numerous investigations and the many hundreds of volunteers who participated in these studies.

Misleading: Introduction, “Genetic Toxicity,” Page 23-24

The statement, “There is little published mutagenicity data for aspartame,” is incomplete and misleading. Studies demonstrating the lack of mutagenicity for aspartame were published by JECFA (WHO, 1980) and included results from a number of different systems evaluating mutagenicity including *Salmonella* mutagenicity assays with and without metabolic activation in test strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100, the Dominant Lethal assay, *in vivo* cytogenetic studies, and in the Host mediated assay. Mutagenicity data for aspartame are also discussed in Molinary (1984), Kotsonis and Hjelle (1996) and Butchko et al. (2002). All safety data on aspartame, including entire studies and all data, are publicly available upon request from the FDA.

In consideration of the respectfully submitted comments above, these reviewers look forward to a scientifically balanced NTP Final Report and the publication in the peer-reviewed scientific literature of the important results from these aspartame studies in three strains of transgenic mice. We follow progress in the process of finalization of NTP Technical Report GGM 1 and peer-reviewed publication of these results with keen interest.

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